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FINAL REPORT ON  
GRANT  
CONTRACT NO DA -CRD-AG-S92-544-64-G27

INCLUSIVE DATES 17 Sept. 1964 TO 16 Sept. 1965

AD 474986

## SUBJECT OF INVESTIGATION

NATURAL DRUG RESISTANCE OF HETEROGENEOUS

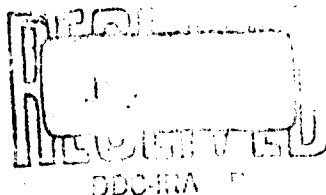
SINGLE CELL TRANSPLANTS

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## ABSTRACT

### NATURAL DRUG RESISTANCE OF HETEROGENEOUS SINGLE CELL TRANSPLANTS

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Population analysis of a cancer clone derived from a single cancer cell was made, using a clonal transplant-strain of the rat ascites hepatoma, CL-1-AH-66F. The natural resistance to nitrogen mustard N-oxide and the chromosomal constitution were studied, after establishing subclonal tumors from the original clone CL-1-AH-66F. The results indicated the following facts:

1. The cancer clone is not a uniform but a complex population of cells with different degrees of drug resistance.
2. The degree of drug resistance of the original clone and its subclones varies naturally within a certain range during serial passages of them in compatible hosts.
3. No specific correlation was found between the degree of drug resistance and chromosomal feature of the subclones.
4. It was suggested that the variation in drug resistance of cancer clone is not always associated to the genetic heterogeneity of cells in the cancer clone.

**NATURAL DRUG RESISTANCE OF HETEROGENEOUS  
SINGLE CELL TRANSPLANTS**

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NATURAL DRUG RESISTANCE OF HETEROGENEOUS SINGLE CELL  
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TO NITROGEN MUSTARD N-OXIDE AND CHROMOSOMES  
OF CELLS WITHIN A CLONAL CANCER  
CELL POPULATION

I. INTRODUCTION

A cancer is a single clinical entity, but there is a considerable degree of variation among its cell population. Chromosomal variations have been demonstrated by many karyologists. No single chromosome number is absolutely predominant in a cancer cell population. Even cells of a single-cell-derived cancer clone differ from each other in their chromosomal features, and stable polyploid clones have been isolated from predominantly near-diploid cancer cell populations.<sup>3)</sup>

Cells within a cancer differ from each other in their degrees of natural resistance to nitrogen mustard N-oxide ( $\text{HN}_2$  N-oxide) and the degrees vary naturally around a certain level during animal passages of that tumor.<sup>5)</sup> The term "natural resistance" is used here to denote the drug resistance which the tumor shows at its onset, in contrast to "acquired" or "induced" resistance which develops following repeated contact of the tumor cells with the drug.

The acquired or induced drug resistance of some tumors is accompanied by chromosomal changes of their cells.<sup>1)</sup> However, the visible chromosomal change does not always reflect the altered drug resistance. A near-tetraploid clone isolated from the Yoshida sarcoma, a predominantly near-diploid tumor cell population, showed the same degree of resistance to nitrogen mustard ( $\text{HN}_2$ ) as the parental population.<sup>6)</sup>

The present study attempts to demonstrate;  
a) whether cellular constitution of a single-cell-derived clonal cancer cell population is uniform as regards the degree of natural drug resistance;  
b) whether the degree of natural drug resistance

of the cancer clone varies during animal passages;  
and  
c) whether any correlation is found between the degree of natural drug resistance and chromosomal features of cells in the cancer clone.

## II. MATERIAL AND METHOD

### 1. CL-1-AH-66F, a clonal tumor.

A clonal subline tumor of the ascites hepatoma AH-66F of the rat, designated as CL-1-AH-66F, was employed. This clone was originally established by transplantation of a single cell of the AH-66F and then maintained by serial passages in "Donryu" rats. The CL-1-AH-66F showed a uniform growth and 100% lethal takes in the rats. Morphologically, the CL-1-AH-66F is a free cell tumor as seen from Figure 1

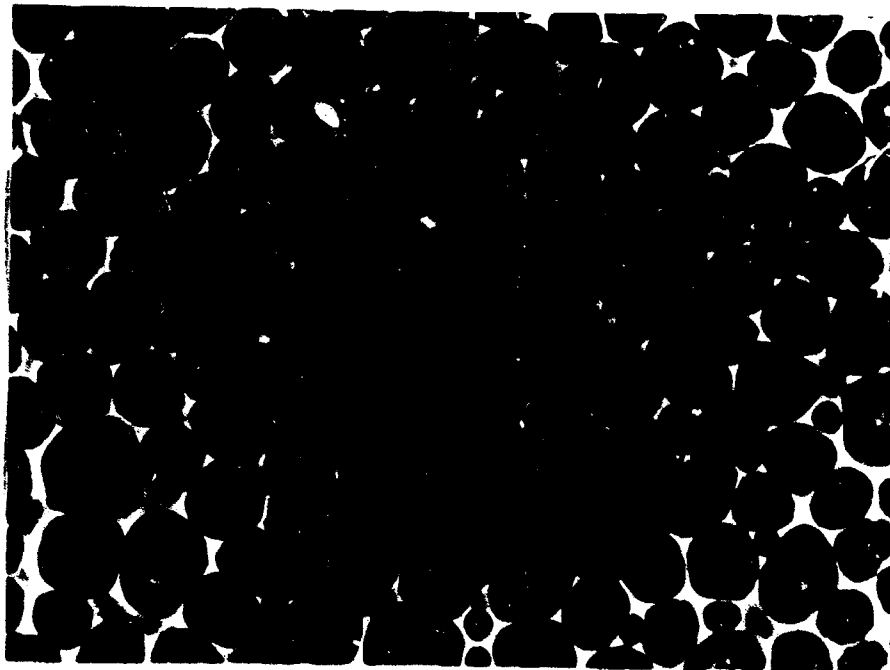


Figure 1. Phase-contrast microscopic view of a single-cell-derived cancer clone, CL-1-AH-66F. Individually isolated hepatoma cells. 51st transplant generation

and the tumor cells do not make cell-clusters or -bonding in ascitic fluid but they proliferate as individually isolated single cells. The resistance to  $\text{HN}_2$  N-oxide and chromosomes of the CL-1-AH-66F were examined using the ascites of the 53rd transplant generation, and every 10th subsequent transplant generation.

## 2. Establishment of subclonal tumors from CL-1-AH-66F.

Single cell transplantations were carried out, using the ascites of the CL-1-AH-66F. At the time of the transplantation, the clone CL-1-AH-66F was between 51st to 52nd serial transplant generation. The ascites including well-proliferated tumor cells was removed from the rat 4 days after intraperitoneal transplantation with  $10^7$  CL-1-AH-66F cells. The ascites was diluted with a mixture of equal amount of horse serum and Hanks' balanced saline solution to such a degree that a droplet of this diluted ascites might contain only one or a few cells. The degree of dilution varied with different density of tumor cells in the original ascites, but it was always over 50,000 times. A droplet, confirmed microscopically as including only one cell, was sucked into a glass microcapillary by the aid of micromanipulator and injected intraperitoneally into a normal rat. Ascitic fluid of the injected animal was examined every 4 days after the injection as a rule. When the transplantation was successful and the cell was found actively multiplying,  $10^6$  cells were transplanted into each rat of the groups serving to examine natural drug resistance and chromosomal features. A total of 100 single cell transplantations was carried out.

## 3. Examination of natural drug resistance

$\text{HN}_2$  N-oxide, methyl-bis- $\beta$ -chloroethylamine N-oxide, causes a characteristic cytological effect on ascites tumor cells. Scattering, coagulation and laceration of chromosomes, chromosome-bridge and formation of giant tumor cells as well as their necrosis take the majority of the effect. These changes are very conspicuous so that the changes were used for the determination of grade of resistance.<sup>5)</sup> The procedure was as follows.

The rats bearing the clone CL-1-AH-66F or its subclones, were injected intraperitoneally with various doses of HN<sub>2</sub> N-oxide 3 days after transplantation with 10<sup>7</sup> tumor cells. The injected drug will come in contact in the same way with all of tumor cells floating in ascites. The ascites of injected rats were microscopically examined for the cytological effect caused by the drug using Giemsa-stained smears every 24 hours until 96 hours after the drug injection. The procedure was repeated and the minimum dose of the drug required to induce the cytological effect on one-half of all tumor cells present was determined and designated as the minimum effective dose -- MED. The MED was represented as indicator of the degree of natural resistance to the drug. The larger the MED of a tumor is, the more resistant to the drug the tumor is, and vice versa.

#### 4. Examination of chromosomes.

Colchicine-treated 4-day-old tumor ascites of the original clone CL-1-AH-66F or its subclones were used. Five hours before the ascites sampling, each of the tumor animals was injected intraperitoneally with colchicine dissolved in physiological saline, in a dose of 0.035 mg/kg of body weight. The ascites obtained was mixed in a test tube with 0.04 mol. NaCl solution kept at 37°C. Ratio of the ascites to the NaCl solution was 1:20. After shaking the tube vigorously, it was kept at 37°C for about 10 minutes. The fluid was centrifuged and the supernatant was decanted. The sedimented tumor material was stained by addition of aceto-orcein solution of about 5 volumes of the tumor material, stirring with a glass rod. A drop of the material was placed on a slide glass, covered with a cover slip under pressure and sealed with balsam-paraffin. Fifty well-spread metaphase plates were observed for each preparation. Magnification used for the examination was 1,000X. All the observed metaphase chromosomes were drawn and photographic records were made. The number of chromosomes and their morphological characteristics were studied.

#### 5. Serial transplantations of the clone CL-1-AH-66F and its subclones.

The original clonal tumor CL-1-AH-66F and its 6 subclonal tumors have been successively transplanted in Donryu rats. The number of inoculated cells was

$10^7$  at every transplantation. The 6 subclones used were those with different MED and/or ploidy as described later. They were selected from all the subclones developed by the single cell transplantations. The MED and chromosomes of these 6 subclones were studied at every 10th transplant generation and compared with each other and with those of the original clone CL-1-AH-66F. Throughout these experiments, male Donryu rats weighing about 100g were employed. The animals are genetically high homogeneous strain of rat<sup>7)</sup> and their susceptibility to the transplantation of CL-1-AH-66F cells was 100%, as stated above. They were housed in metal cages, 5 to a cage. The semisynthetic cube diet (CE-2, Central Lab. Exp. Animals, Tokyo) and water were given ad libitum.

### III. RESULT

#### 1. Natural resistance to $\text{HN}_2$ N-oxide of clone CL-1-AH-66F and its subclones.

An ascites tumor developed in 44 of 100 rats which received a single CL-1-AH-66F cell intraperitoneally. Twenty-eight of these 44 subclonal tumors were examined for their MEDs. Results of this examination revealed that 5 types of subclones which differed in the MED had been derived from the single original transplanted cells (Table 1). One of the

Table 1

Natural Resistance to  $\text{HN}_2$  N-oxide of Subclonal Tumors from Clone CL-1-AH-66F

No. of subclones	MED of $\text{HN}_2$ N-oxide (mg/kg)
2	10.0
1	5.0
11	1.0
12	0.5
2	0.1
CL-1-AH-66F, parental clonal cell population	1.0

Examinations: CL-1-AH-66F, 53rd gen.; Subclones, 1st gen.

types of the subclones showed the same MED as the original clone CL-1-AH-66F, while the remaining 4 types of the subclones had MEDs which were higher or lower as compared with that of the original clone. This means that the clonal tumor CL-1-AH-66F is a complex of at least 5 kinds of cells regarding the degree of natural resistance to  $\text{HN}_2$  N-oxide. Therefore, the drug resistance of the clone is the averaged sum of that of all its constituents with different degrees of resistance.

2. Variation in natural resistance to  $\text{HN}_2$  N-oxide of clone CL-1-AH-66F and its 6 subclones during serial animal transfers.

Repeated examinations for the MED of clone CL-1-AH-66F and its 6 subclones were performed at different transplant generations. Results are indicated in Table 2. The MED of the parental clone was 1.0

Table 2

Natural Resistance to  $\text{HN}_2$  N-oxide of Clone CL-1-AH-66F its 6 Subclones during Serial Animal Passages

Generation	MED (mg/kg)					
	1st	10th	20th	30th	40th	50th
Subclone-1	10.0	5.0	5.0	5.0	0.5	1.0
Subclone-2	5.0	1.0	0.5	1.0	0.5	1.0
Subclone-3	1.0	1.0	1.0	1.0	5.0	0.5
Subclone-4	1.0	1.0	1.0	1.0	0.5	0.5
Subclone-5	0.5	1.0	1.0	1.0	1.0	0.5
Subclone-6	0.1	0.5	1.0	1.0	1.0	1.0
CL-1-AH-66F parental clone (gen.)	1.0 (53)	5.0 (62)	0.5 (72)	1.0 (82)	1.0 (92)	1.0 (102)

at the first examination. Although it varied upwards and downwards during the subsequent 20 passages it settled at the MED level of 1.0 at the latest 30 transplant generations. The MEDs of more resistant subclones, i.e. Subclone-1 and Subclone-2, decreased

gradually whereas those of less resistant ones, i.e. Subclone-5 and Subclone-6, increased with repeated animal passages. The remaining 2 subclones, Subclone-3 and Subclone-4, also varied in their MEDs through the passages. All these subclones reached 1.0 or 0.5 MED level at the 50th transplant generation. This means that the MEDs of the original clone and subclones naturally vary during animal passages around the MED level of 1.0 of the original clone and even the subclones whose MEDs deviated at first far upwards or downwards from the MED of the original parental clone showed later the MED level of the original clone. The range of variation was evidently within the MED levels 0.1-10.0 shown by different subclones when established from the original clone CL-1-AH-66F.

### 3. Chromosome features of clone CL-1-AH-66F and its 6 subclones.

The chromosome numbers of the original clone and its subclones are shown in Figure 2. A slight

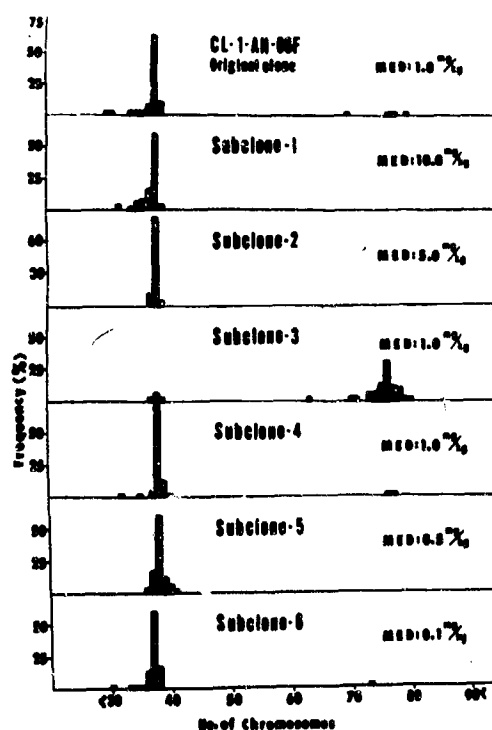


Figure 2. Distribution of chromosome number and MED of original clone CL-1-AH-66F and its 6 subclones.

Examinations: CL-1-AH-66F, 53rd gen.; Subclones, 1st gen.

variation with the modal peak is seen in the distribution of chromosome number. The modal chromosome number of 4 subclones, i.e. Subclone-1, Subclone-2, Subclone-4 and Subclone-5, was 38, a near-diploid number; the same number as that of the original clone. The modal chromosome number of Subclone-3 was 76, just double the number of the modal chromosome number of the original clone, while that of Subclone-6 was 37, the number differing very slightly but distinctly from the modal chromosome number of the original clone.

Figure 3 shows typical metaphase chromosomes

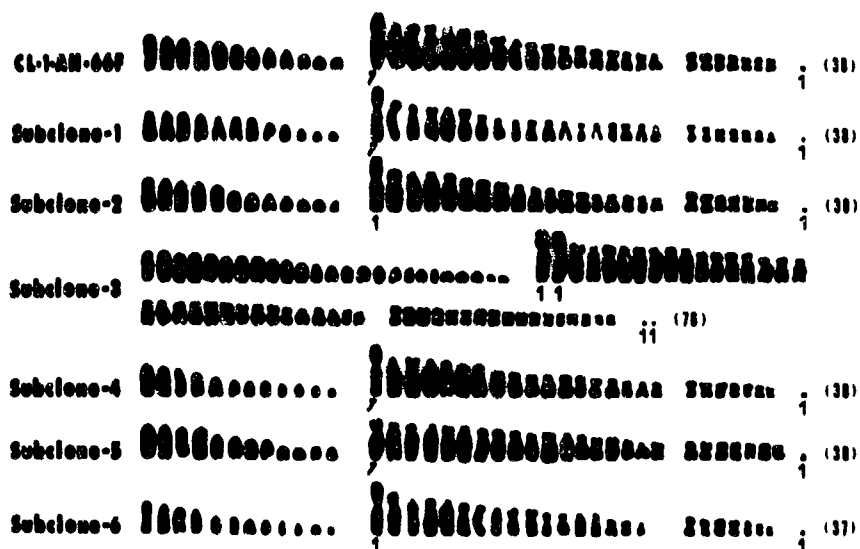


Figure 3. Typical metaphase chromosomes of the original clone CL-1-AH-66F and its 6 subclones. Chromosomes were arranged from left to right in order of decreasing length in groups of the same types, telocentric, submeta- or subtelocentric, metacentric and minute ones. Markers are indicated by the symbol / . Numbers of chromosomes are shown in parentheses.

Examinations: CL-1-AH-66F, 53rd gen.; Subclones, 1st gen.

which are most frequent in the modal regions of the original clone and its 6 subclones. The chromosomes were classified, according to the location of the constriction or size, into 4 types, telocentric (T), metacentric (M), subtelocentric or submetacentric (S) and minute (m) chromosomes. The length ratio of both arms of M chromosome ranged from 1:1 to 1:1.3. All the chromosomes were arranged in the order of decreasing size in groups of the same type. The number of each type of chromosomes varied slightly even in the modal idiogram, but the most frequent pattern is indicated in Figure 3.

Morphologically, not all the chromosomes in each of the present near-diploid tumors could be brought into pairs. Two kinds of chromosomes with conspicuous shape and size were noticed. One of the largest S (submetacentric) and one m chromosome, shown by the symbol 7 in Figure 3, were present in the chromosomes of the original clone. These characteristic chromosomes present "markers" for the chromosomal pattern of CL-1-AH-66F. The markers were also detected in each subclone. Subclone-3 cells with doubled the number of chromosomes of the original clone had 2 of each marker chromosome.

The chromosome number and chromosomal pattern of 4 subclones, i.e. Subclone-1, Subclone-2, Subclone-4 and Subclone-5, showed complete conformity to those of the original clone. Therefore, it is evident that those 4 subclones were derived respectively from single cells of the modal area of the original clone. It is unlikely, however, that Subclone-3 with the modal chromosome number of 76 is a descendant of a single polyploid cell of the original clone. In the population of Subclone-3 there are cells of 38 chromosomes, though they are less frequent, which are quite identical to those of the modal region of the original clone. It is difficult to believe that during mitotic division of a single original tetraploid cell it produces diploid cells with just one-half the chromosome composition of the original polyploid. On the other hand, it is a well-known fact that tetraploid cells develop from diploid cells. This would suggest that the near-diploid cells in Subclone-3 were not derived from a near-tetraploid cell of the original clone. Near-tetraploids predominating in Subclone-3 could be descendants which occurred during proliferations of the original transplanted near-diploid cell.

The chromosome pattern of Subclone-6 showed that a small S or M element is lacking compared to the set of 38 chromosomes of the original clone. This type of chromosome pattern was detected in less than 10% in the original clone of the 53rd generation.

As seen from Figure 2, 3 subclones, Subclone-1, Subclone-2 and Subclone-5, whose chromosomal patterns are the same as those of the original clone, differed in MED of  $\text{HN}_2$  N-oxide from the other subclones and the original clone. Subclone-3 and Subclone-4, which showed the same MED as the original clone, differed in ploidy. This means that the chromosomal feature of tumors have no special relationship to their degree of drug resistance. Subclone-6 differed in both chromosomal pattern and MED from its original clone.

#### 4. Variations in chromosomal features and MEDs of clone CL-1-AH-66F and its 6 subclones.

Results of the repeated examinations of chromosomes of each tumor line are indicated in Figure 4.

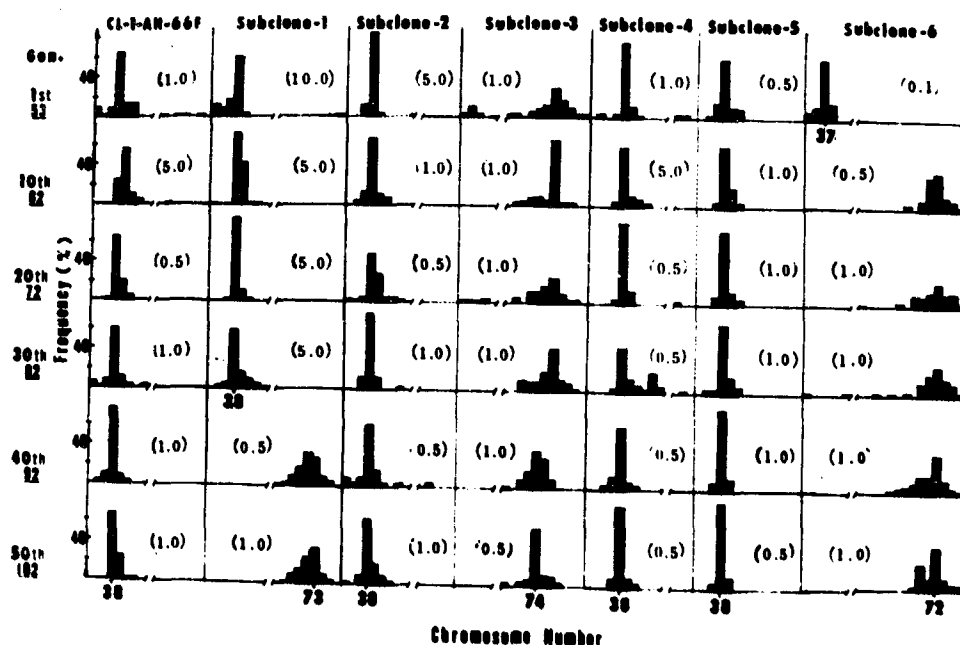


Figure 4. Distribution of chromosome number and MED of original clone CL-1-AH-66F and its 6 subclones at different transplant generations of animal passages. Number in parentheses shows MED. Underline number indicates transplant generation of the original clone.

The modal chromosome number of the original clone was 38 at the first examination as stated already, but a very slight and temporary shift from 38 to 39 in the modal number was found 9 passages later, then the number again fell to 38 and was stable thereafter. The modal chromosome number of Subclone-1 stayed at 38 for 30 passages but then changed to 73 where it remained through the 50th transplant generation. No variations were detected in the modal chromosome numbers of Subclone-2, Subclone-4 and Subclone-5. The modal chromosome number of Subclone-3 was 76 and it remained at this level during 30 animal transfers, but decreased to 74 at the 40th transplant generation where it remained at the 50th transplant generation. In the remaining Subclone-6 the modal chromosome number was first 37, but then it rose to 73 at the 10th generation and persisted to the 40th transfer of this subclone, and then decreased to 72 at the 50th transplant generation.

Comparative studies of modal chromosome patterns demonstrated that no marked variations occurred in Subclone-2, Subclone-4 and Subclone-5 during their 50 passages. As to the remaining subclones and the original clone, the following findings were obtained.

(1) Original clone, CL-1-AH-66F. The shift from 38 to 39 in the modal chromosome number was reflected by one small S or M type chromosome in addition to the original set of 38 modal chromosomes of the 53rd transplant generation. The same pattern of 39 elements was also found in about 8% of the metaphases of the 53rd generation. Even when the modal chromosome number was 39, cells with the original set of 38 elements were detected in about 25% of the cells and such cells were in the majority again in later transfers of the original clone.

(2) Subclone-1. The modal pattern of 38 chromosomes persisted during 30 transfers. The modal chromosomes in the 40th and 50th generations showed that T, M and m elements doubled in number, compared to the original set, while the number of S chromosomes was less than the expected double number. There was a 2-fold increase in the S and m marker chromosomes.

(3) Subclone-3. The modal chromosome number of 76, in the first 30 transplant generations was reflected by the presence of each element in just the double the number of chromosomes as in the original clone. The shift from 76 to 74 was seen with the decrease of small S or M elements.

(4) Subclone-6. The modal number of chromosomes was

37 at first. The modal chromosome pattern in the 10th to the 40 generations revealed almost twice the number of each type of the 37 chromosomes with only one deficient small S or M chromosome. In the 50th transfer, one more element of small S or M was lost.

The modal chromosomes of the 6 subclones of the 50th generation are shown in Figure 5.

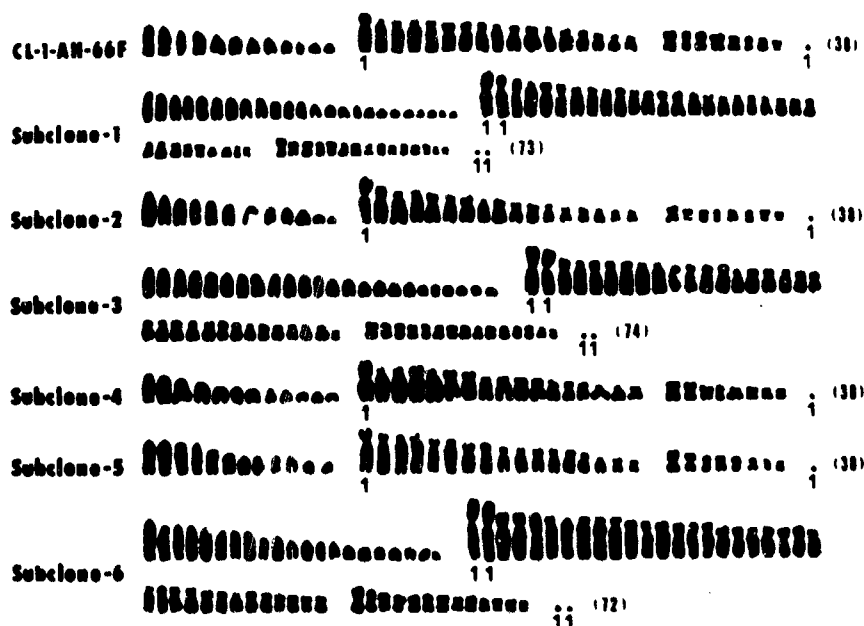


Figure 5. Typical metaphase chromosomes of the original clone CL-1-AH-66F and its 6 subclones. Compare with Figure 3 and note the difference in chromosomal patterns of Subclone-1, Subclone-3 and Subclone-6.

Examinations: CL-1-AH-66F, 102nd gen.; Subclones, 50th gen.

Polyploid cells where occur in the near-diploid cell population become the predominant cell type occasionally. However, it seems to be rare that near-tetraploids with exact doubled sets of the original

near-diploid chromosomes predominate in a cancer cell population for long periods of animal passages. As seen in the case of Subclone-3, the observed ploidy change may be expressed as  $2n' \rightarrow 4n' \rightarrow 4n'-x$ , when the original pattern of chromosomes shown by  $2n'$ . Similar variations might be possible for the development of near-tetraploid Subclone-1 and Subclone-6, although the modal predominance of cells with just 76 or 74 chromosomes -exact doubling of chromosomes of the onset of these subclones, was not encountered by the present limited number of chromosome examinations at intervals.

Comparative studies of MEDs and chromosome features of each tumor line at various transfer generations disclosed that there are no specific correlations between them. The degree of drug resistance and chromosomal features in cancer clone can vary independently.

5. Growth characteristics of clone CL-1-AH-66F and its 6 subclones.

Transplantability and host survival time of these clonal and subclonal tumors showed a considerable similarity (Table 3). The incidence of lethal "takes"

Table 3

Transplantability and Survival Time of Hosts of CL-1-AH-66F and its 6 Subclones

Tumors	Generations	No. of Recipients	Takes	% Med. Surv. time in days
CL-1-AH-66F	53-102	98	98	100.0 9
Subclone-1	1-50	98	98	100.0 9
Subclone-2	1-50	96	96	100.0 9
Subclone-3	1-50	94	94	100.0 9
Subclone-4	1-50	93	93	100.0 9
Subclone-5	1-50	97	97	100.0 9
Subclone-6	1-50	96	96	100.0 10

was 100% for each tumor line. Median survival time of the hosts was 9 days for the original clone and for the 5 subclones, while the survival time of the

remaining Subclone-6 was 10 days. Any significant differences in the survival time were not detected before and after chromosomal changes of Subclone-1 and Subclone-6. The ascitic picture of each tumor line was also quite similar. It was characterized by individual proliferating tumor cells, however, the size of the living tumor cells varied with the cell strains. Cells of near-tetraploids such as Subclone-1, Subclone-3 and Subclone-6, were larger than those of near-diploids, i.e. the original clone, Subclone-2, Subclone-4 and Subclone-5 (Table 4).

Table 4

Diameter of Tumor Cells

Tumors	Diameter in $\mu$ (M. $\pm$ S.D.)
CL-1-AH-66F (orig. clone)	14.1 $\pm$ 1.3
Subclone-1	16.9 $\pm$ 0.7
Subclone-2	13.6 $\pm$ 1.3
Subclone-3	18.0 $\pm$ 1.5
Subclone-4	13.5 $\pm$ 1.1
Subclone-5	14.7 $\pm$ 1.1
Subclone-6	17.4 $\pm$ 1.4

Measured on living 100 cells of fresh 4-day-old ascites in a chamber with a depth of 0.1 mm, using NIKON filar-micrometer.

The ascites of each tumor line was accompanied by hemorrhage usually 5 days after the intraperitoneal transplantation when small local invasions in various peritoneal tissues were macroscopically detected. Distant metastases were frequently seen in the mediastinal lymph nodes and sometimes in the lungs. No marked difference could be found in the incidence and/or degree of local invasion and metastases of the 7 tumor lines.

#### IV. COMMENT

The present study shows the cellular variability of a single-cell-derived cancer clone. This can be concluded from the fact that cells of a clone differ from each other in their natural resistance to  $\text{HN}_2$  N-oxide. Therefore, the drug resistance of the clone is the averaged sum of that of all its constituents with different degrees of resistance. It is said that comparable and chromosomally-different cancer clones differ from each other in their physiological and immunological characteristics.<sup>2)</sup> However, it was impossible in this study to find out any clear correlation between the degree of natural resistance to  $\text{HN}_2$  N-oxide and chromosomal features of the present subclones; MED varied even if the chromosomal feature was the same and vice versa, the MED was similar when the ploidy was different.

The drug resistance and chromosomal constitution during serial animal passages of the original clone and its subclones in compatible rats were examined repeatedly. The results showed that the  $\text{HN}_2$  N-oxide MED varied within a certain range but there was a tendency for the high or low MED to approach that of the modal MED of the original clone. Thus, the variation in  $\text{HN}_2$  N-oxide resistance could not be the result of a stable, genetic change of cells but of a physiological or adaptive change. Occurrence of varied MEDs may be explained as the result of variation of the frequency ratio of the cells with fluctuated and different resistance in the clonal and subclonal populations during animal passages.

It was also demonstrated that the shift from the near-diploid to the near-tetraploid in chromosomes occurred in 3 subclones. The ploidy variation could be expressed as  $2n' \rightarrow 4n' \rightarrow 4n'-x$ . Similar variations have been observed in different rat ascites tumors.<sup>4)</sup> During animal passages of the original clone, a slight shift was found in the modal chromosome number. This change was transient, however, reverting back to the original modal number.

Generally, it can be said that near-tetraploid tumor cells arise from near-diploid tumor cells and some of the polyploids predominate the tumor cell population in certain hosts. This phenomenon, so far as observed till now, is a stable change during serial transplant generations, although slight variations in the chromosome number from  $4n'$  were found. It was

difficult to ascertain the significance of these changes on inheritable characteristics of these malignant cells. On the other hand, the transient slight variation in the modal chromosome number in the CL-1-AH-66F clone was an interesting fact. Its mode of appearance or significance can not be explained, although it may be due to the development of variant with higher growth advantage. Another possibility may be that the change was the result of altered host conditions. Of course both mechanisms may not be mutually exclusive. Similar explanations may be required for the fact of establishment of Subclone-6 with the modal chromosome number of  $2n'-1$ , 37, from the original clone with the modal chromosome 38.

No significant differences were detected in general biological characteristics such as transplantability, life span of hosts, local invasion, metastatic ability, etc., among the original clone and its subclones.

#### V. SUMMARY AND CONCLUSION

Population analysis of a cancer clone derived from a single cancer cell was made, using a clonal transplant-strain of the rat ascites hepatoma, CL-1-AH-66F. The natural resistance to nitrogen mustard N-oxide and the chromosomal constitution were studied, after establishing subclonal tumors from the original clone CL-1-AH-66F. The results indicated the following facts:

1. The cancer clone is not a uniform but a complex population of cells with different degrees of drug resistance.
2. The degree of drug resistance of the original clone and its subclones varies naturally within a certain range during serial passages of them in compatible hosts.
3. No specific correlation was found between the degree of drug resistance and chromosomal feature of the subclones.
4. It was suggested that the variable drug resistance of cancer clone is not always associated to the genetic heterogeneity of cells in the cancer clone.

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DOCUMENT CONTROL DATA - R&D		
(Security classification of title, body of abstract and indexing annotation must be entered when the original report is classified)		
1. ORIGINATING ACTIVITY (Corporate author) The Medical Institute of Sasaki Foundation, Tokyo (Japan)		2a. REPORT SECURITY CLASSIFICATION Unclassified
		2b. GROUP
3. REPORT TITLE NATURAL DRUG RESISTANCE OF HETEROGENEOUS SINGLE CELL TRANSPLANTS (U)		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Final Report, 17 Sept 1964 - 16 Sept 1965		
5. AUTHOR(S) (Last name, first name, initial) Yoshida, Tomizo		
6. REPORT DATE 29 September 1965	7a. TOTAL NO. OF PAGES 17	7b. NO. OF REFS 7
8. INDEXING GRANT NO. DA-CRD-AG-S92-544 54-G27	9a. ORIGINATOR'S REPORT NUMBER(S) J-202	
9. PROJECT NO. 2N014501B71D	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
10. Task OO 014FE		
10. AVAILABILITY/LONG-TERM SERVICES Qualified requesters may obtain copies of this report from DDC.		
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY U. S. Army R&D Group (Far East) APO San Francisco 96343
13. ABSTRACT Population analysis of a cancer clone derived from a single cancer cell was made, using a clonal transplant-strain of the rat ascites hepatoma, CL-1-AH-66F. The natural resistance to nitrogen mustard N-oxide and the chromosomal constitution were studied, after establishing subclonal tumors from the original clone CL-1-AH-66F. The results indicated the following facts: 1. The cancer clone is not a uniform but a complex population of cells with different degrees of drug resistance. 2. The degree of drug resistance of the original and its subclones varies naturally within a certain range during serial passage of them in compatible hosts. 3. No specific correlation was found between the degree of drug resistance and chromosomal feature of the subclones. 4. It was suggested that the variation in drug resistance of cancer clone is not always associated to the genetic heterogeneity of cells in the cancer clone. (Author)		

DD FORM 1473

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KEY WORDS	LINK A		LINK B		LINK C	
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Cell transplantation Cytology Drug resistance Neoplasms Microphotography Pathology Japan						

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